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EFFECTS OF MIREX AND METHOXYCHLOR ON STRIPED MULLET, 'mugil cephalus L'

Jong H. Lee, et al

Oceanic Institute

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The effects of two chlorinated insecticides, mirex and methoxychlor, on striped mullet, Mugil cephalus L., were studied. Test concentrations of both insecticides used were 0.01, 0.1, 1.0 and 10.0 ppm in dynamic bioassay. Young juveniles were more susceptible to mirex exposure than older juveniles or adults. No mortalities occurred in older juveniles and adults exposed to mirex for 96 hours. For young juveniles, mortalities were highest in concentrations of 0.1 and 1.0 ppm and were less in concentrations of 0.01 and 10.0 ppm. Significant amounts of mirex residues were accumulated in the body tissues of the test fish; concentrations increased with increased environmental concentrations. Methoxychlor was more toxic to mullet than mirex. Mortalities were greater than 90 percent over a 96-hour period for all life stages studied at concentrations of 0.1, 1.0 and 10.0 ppm. Mortality at a concentration of 0.01 was 5.1 percent or less for 96 hours. Relative to mirex, small amounts of methoxychlor residues accumulated in the tissues of the test fish. Results of the experiments on eggs and larvae were inconclusive. Egg survival was slightly better in mirex than in methoxychlor over a 96-hour period. Larval survival was generally better in mirex than methoxychlor.

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EFFECTS OF MIREX AND METHOXYCHLOR ON STRIPED MULLET, Mugil cephalus L.

by

Jong H. Lee, Colin E. Nash and Joseph R. Sylvester

Project Officer
David J. Hansen
Gulf Breeze Environmental Research Laboratory
National Environmental Research Center
Sabine Island
Gulf Breeze, Florida 32561

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ABSTRACT

The effects of two chlorinated insecticides, mirex and methoxychlor, on eggs, larvae, juveniles, and adults of the striped mullet, Mugil cephalus L., were studied. Test concentrations of both insecticides used were 0.01, 0.1, 1.0 and 10.1 mg/l in dynamic bioassays of juveniles and adults and static bioassays of eggs and larvae.

Young juveniles, standard length 20 - 43 mm, were apparently more susceptible to mirex exposure than older juveniles, standard length 70 - 150 mm, and adults, standard length 260 - 380 mm. No mortalities occurred in older juveniles and adults exposed to mirex for 96 hours. Young juveniles exposed to 0.1 and 1.0 mg/l of mirex had higher mortality rates (27 and 32 percent) than control fish, but mortalities of 0.01- and 10.0-mg/l exposed fish were not different from controls. Significant amounts of mirex residues were accumulated in the body tissue of the test fish. Juveniles and adults exposed to 0.01 mg/l mirex were found to accumulate 0.2 and 0.4 μg/g respectively during the bioassays. The concentrations in fish increased with increasing mirex concentrations in test water. The highest accumulation was found in the adult fish exposed to 10.0 mg/l (37.3 μg/g).

Methoxychlor was more toxic to mullet than mirex. Mortalities were greater than 90 percent over a 96-hour period for juvenile and adult fish at concentrations of 0.1, 1.0 and 10.0 mg/l. Methoxychlor residues accumulated in the tissues of test fish in smaller amounts than mirex in mirex-exposed fish. Young adults and juveniles exposed to 0.01 mg/l methoxychlor accumulated 0.1 and 0.2 μg/g respectively. The accumulated amounts in 10.0 mg/l concentration were 1.7 μg/g for young juveniles and 11.1 μg/g for adult fish.

Results of the experiments on eggs and larvae were inconclusive because of the natural high mortality of both stages in culture conditions. Egg and larval survival was generally better in mirex than in methoxychlor over a 96-hour period.

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SECTION I

CONCLUSIONS

1. Young juvenile mullet (20–43 mm standard length) were apparently more susceptible to mirex exposure than older juveniles (70–150 mm standard length) or adults (260–380 mm standard length).

2. Mirex was accumulated by mullet, and body concentrations in whole fish (wet weight) increased with increased concentration in test water, which indicates that the insecticide is stable in striped mullet.

3. Mirex was accumulated most (21.5 μg/g) in visceral organs and least (1.4 μg/g) in skin and muscle of adult mullet for a 96-hour exposure period. Gills and hearts accumulated 2.0 μg/g for the same exposure time.

4. Methoxychlor was more toxic to mullet than mirex at concentrations of 0.1, 1.0 and 10.1 mg/l over a 96-hour period. However, mortalities of young juveniles in 0.1- and 1.0-mg/l mirex-treated water were 27 and 32 percent respectively, by far the highest among mirex-exposed fish or controls. No mortality occurred among older juveniles and adults exposed to mirex over the same experimental period.

5. Relative to mirex, small amounts of methoxychlor were accumulated in whole juvenile and adult mullet. Test fish exposed to 10.0 mg/l methoxychlor contained 1.7 μg/g for juveniles and 11.1 μg/g for adults over a 96-hour period.

6. Results with eggs and larvae were inconclusive because of the natural high mortality of both stages in culture conditions. Eggs and larvae from the same broodstock had a better survival rate in mirex than in methoxychlor.

SECTION II

INTRODUCTION

Mirex, a polycyclic chlorocarbon insecticide (dodecachloroocta-hydro-1,3,4-metheno-2H-cyclobuta[cd] pentalene), has been used many years for control of the imported fire ant (Solenopsis savissima richteri) in various southeastern states (Coon and Fleet, 1970). Accumulation of mirex residues by various species of aquatic organisms (Butler, 1969; Wolfe and Norment, 1973; Borthwick et al., 1973) is indicative of the widespread occurrence and extent of environmental contamination by this insecticide. The toxicity of mirex to non-target organisms has been reported by a number of workers on selected freshwater fishes (Van Valin et al., 1968), estuarine and freshwater invertebrates (Lowe et al., 1971; Naqvi et al., 1973), larval crabs (Bookout et al., 1972), and freshwater crayfish (Ludke et al., 1971).

Methoxychlor (1,1,1-trichloro-2,2-bis[p-methoxyphenyl] ethane) has been a commercial insecticide since discovery of its insecticidal properties (Lauger et al., 1944), and has replaced DDT, especially for the control of blackfly larvae in streams, elm bark beetles, and fruit and garden pests (Burdick et al., 1968; Reinbold et al., 1971). The metabolism of the insecticide by warm-blooded animals is rapid and its toxicity to many insects is similar to that of DDT (Menzie, 1969). The physico-chemical properties of methoxychlor and its toxicology to higher animals indicate that methoxychlor may be one of the safest of all insecticides (Negherbon, 1959).

However, methoxychlor could be hazardous to aquatic life because it accumulates in the environment like other chlorinated insecticides. The toxicity of methoxychlor has been reported for decapod marine crustaceans (Eisler, 1969), selected freshwater fish species, such as fathead minnow, goldfish, guppies, and mummichog (Henderson et al., 1959; Eisler, 1970), bluegills and certain freshwater invertebrates (Kennedy et al., 1970), and tadpoles (Sanders, 1970).

The purpose of this investigation was to determine the acute toxicity and accumulation rates of mirex and methoxychlor to juvenile and adult striped mullet (Mugil cephalus L.) using 96-hour continuous flow bioassays; and to eggs and larvae in 48-hour and 96-hour static bioassays respectively. Striped mullet have an extensive geographic distribution and can tolerate a wide range of physical parameters in both fresh and saltwater environments (Sylvester et al., 1974; Thompson, 1966). The striped mullet are predominantly herbivores and detritivores and a highly desirable food fish in many parts of the world.

SECTION III

EFFECTS OF MIREX AND METHOXYCHLOR ON JUVENILE AND ADULT STRIPED MULLET, Mugil cephalus L.

MATERIALS AND METHODS

Juvenile mullet used in this study were seined from coastal streams and bays around the island of Oahu, Hawaii. Young juveniles ranged in standard length from 20 to 43 mm and older juveniles ranged in standard length from 70 to 150 mm. Adult mullet, standard length 260 to 380 mm, were collected from the island of Hawaii. All fish were transported to the laboratory in aerated tanks and acclimated from ten days to two weeks in a 4,000-gallon vat or in ponds.

Mirex was obtained from the Allied Chemical Corporation and contained a minimum of 95 percent active mirex. Methoxychlor contained a minimum of 88 percent active ingredient and was obtained from the E. I. du Pont de Nemour Company.

BIOASSAY TECHNIQUES

Stock solutions of mirex and methoxychlor were prepared with acetone and stored in two-gallon glass jars. Insecticide stock solutions were siphoned at 0.9 ml/min. into baffled mixing chambers where they mixed with 5-μ filtered seawater (Table 1) flowing at 650 ml/min. The seawater solvent and insecticide in the mixing chamber then flowed into a delivery chamber which supplied the desired concentrations to each test aquarium. Acetone and seawater controls were supplied with the same delivery system. The 20-gallon glass aquaria maintained a constant volume of 55 liters of fluid.

The dosing apparatus and procedures of the test were similar to those described by Burke and Ferguson (1968) and Sprague (1969). Four different concentrations of one insecticide--0.01, 0.1, 1.0 and 10.1 mg/l--were run simultaneously with four replicates of each concentration, four controls containing seawater, and four with seawater and acetone. Total flow rate through the test aquaria was 260 ml/min. of insecticide and seawater. The number of mullet used in each test was 25 for young juveniles, 10 for older juveniles, and two for adults. The test fish were transferred into the aquaria approximately 20 hours after the toxicant started flowing.

Mortalities were recorded daily during a 96-hour exposure period and percent mortality adjusted for control mortality according to the method of Ludke et al.

(1971):

$$M_x = \frac{S_c - S_t}{S_c} \times 100$$

where M_x = percent mortality in insecticides
S_c = percent survival in controls
S_t = percent survival in insecticides.

In accumulation tests of both mirex and methoxychlor on juveniles and adults, identical experimental apparatus were used. Desired concentrations were 0.5 mg/l for mirex and 0.01 mg/l for methoxychlor. A set of five aquaria for one insecticide was used in the continuous-flow tests for 96 hours. The number of mullet used in each aquarium was ten for juveniles and two for adults. A sample of fish was taken from the acclimation tank at the start of the experiment before the rest were put under test (0-hour sample). Further samples were taken at 8, 24, 48, and 96 hours with replicates at each time. Samples for adults were taken 24, 48, 72, and 96 hours after they were transferred to the test aquaria.

Table 1. ANALYSIS OF SEAWATER USED IN THE CONTINUOUS-FLOW BIOASSAY FOR MIREX AND METHOXYCHLOR.

Analysis	Range
Temperature (°C)	23.0 - 25.6
Salinity (°/$_{oo}$)	32.5 - 32.9
pH	7.6 - 8.3
Dissolved oxygen (ppm)	4.5 - 6.5
Phosphate (μ mole)	3.5 - 4.0
Nitrate (μ mole)	40 - 50
Ammonia (μ mole)	1.4 - 2.1
Mirex (μg/l)	ND*
Methoxychlor (μg/l)	ND*

*none detected: less than 0.1 μg/l mirex, 0.8 μg/l methoxychlor.

Dissolved oxygen, temperature and salinity were monitored daily with Model 54 YSI oxygen meter and Bissett-Berman salinometer (Model 6263).

RESIDUE ANALYSIS

At the end of 96 hours, live fish were killed in acetone, then rinsed with acetone and frozen for residue analysis. Dead fish in mirex-treated tanks were

removed as soon as they were observed, then rinsed in acetone and kept in the freezer. Methoxychlor was measured from fish killed by the insecticide and pooled at the end of the bioassays, except those in 0.01 mg/l methoxychlor tanks and controls (1-10 ppm). Frozen samples were minced in a laboratory blender. Approximately 5 grams of the minced sample (duplicates from individual test aquaria) were ground thoroughly with additions of anhydrous sodium sulfate in a mortar and pestle. For fish tested for accumulation rates, skin and muscle, gills and heart, and viscera were separately taken from individuals (10 for juveniles, 2 for adults) and blended samples for each part were used for insecticide analyses.

Insecticide was extracted from the mixture with four portions of 50 ml petroleum ether in a water bath at 40°C and concentrated in 50-ml boiling flasks using a rotary evaporator. The extract was then dissolved in 10 ml of petroleum ether saturated with acetonitrile, transferred into a separatory funnel, and extracted three times with 25-ml portions of acetonitrile saturated with petroleum ether. The acetonitrile extract was then combined in a 500 ml separatory funnel containing 300 ml of 2 percent sodium chloride solution and 50 ml of petroleum ether. The ether extract was filtered through a 2-inch column of anhydrous sodium sulfate in a 150 x 24 mm filter tube and condensed to ca. 5 ml using the evaporator. The residue was transferred into a 30 cm x 15 mm i.d. chromatographic column containing 4 inches of Florisil (60/100 mesh, activated at 130°C) topped with 1/2-inch anhydrous sulfate, eluted with 6 percent diethyl ether in petroleum ether, and reduced to an appropriate volume.

Water samples were collected during tests from aquaria for insecticide analysis. The insecticides in the water samples were extracted with 6 percent diethyl ether/hexane mixture. The extract was then dried with anhydrous sodium sulfate and reduced to an appropriate volume.

Identification and quantitative determination of the insecticides were made by gas chromatography equipped with Ni-63-electron capture detector. Operational conditions of the two U-type glass columns (6 ft x 1/4 in. o.d.) were as follows:

	Column I	Column II
Liquid phase	1.5% SP 2250 / 1.95% SP 2401	4% SE-30 / 6% SP 2401
Solid phase	100/120 mesh Supelcon AWDMCS	80/100 mesh Supelcon AWDMCS
Temperature, °C		
Inlet	215	215
Column	200	200
Detector	275	275
N_2 gas flow, cc/min.		
Carrier	80	80
Purge	20	20

All the organic solvents used were nanograde quality. Sodium chloride, sodium sulfate and glass wool were pre-washed with hexane for the removal of any possible contamination.

RESULTS AND DISCUSSION

The results indicate that young striped mullet juveniles are more susceptible to mirex poisoning than either older juveniles or adults (Table 2). No mortalities occurred among older juveniles or adults in the 96-hour bioassay. Among young juveniles highest mortalities of 32.1 and 26.9 percent (adjusted mortality) occurred in mirex concentrations of 1.0 and 0.1 mg/l. However, mortalities in 10.0 and 0.01 mg/l (9.0 and 6.4 percent) did not much differ from those in controls. The causes of the anomaly in mortality between extreme concentrations of 10.0 and 0.01, and the intermediate ones of 1.0 and 0.1, are obscure. The behavior of the young juveniles before death was similar in all test tanks and control tanks.

The anomaly in mortality of juvenile striped mullet could result from a specific action of mirex on the species and the physical parameters of the experimental conditions. Inconsistencies in the effects of mirex on other fishes have been reported elsewhere. In a study of the effects of mirex on bluegill, Lepomis machrochirus, and goldfish, Carrasius auratus, Van Valin et al. (1968) reported that bluegill exposed to 0.0013 mg/l and 1.0 mg/l showed no relationship between mortalities and mirex exposure. However, they observed 68.8 percent mortality in goldfish in 0.1 mg/l, and 85.5 percent mortality in 1.0 mg/l during a 308-day experimental period.

The results of the mirex residue analyses and the measured concentrations of mirex in water are presented in Table 3. The largest amounts of mirex were found in the adults, possibly because adults may have a higher proportion of body fat than juveniles. Amounts of mirex in fish increased with increasing insecticide concentration in test water. An intensive study of the mirex toxicity to estuarine organisms by Lowe et al. (1971) showed that juvenile pinfish, Lagodon rhomboides, concentrated up to 40 mg/kg of mirex in their tissues when fed food having 20 mg/kg mirex with the weekly addition of fire ant bait to the aquaria over a period of five months.

Adult mullet, 278-310 mm in standard length, were exposed to 0.5 mg/l of mirex in water for 96 hours in a continuous-flow system. Organs from various parts of the test mullet were analyzed for mirex accumulation (Table 4). The results indicate that mirex was concentrated most in visceral organs and least in skin and muscle parts. Amounts of mirex found in 96-hour samples were 21.5 μg/g from viscera, 2.0 μg/g from gills and heart, and 1.4 μg/g from skin and muscle.

Table 2. PERCENT MORTALITY OF STRIPED MULLET EXPOSED TO MIREX FOR 96 HOURS IN A CONTINUOUS-FLOW BIOASSAY. FOUR REPLICATE AQUARIA WERE USED FOR EACH CONCENTRATION AND CONTROL. NUMBER OF FISH TESTED PER REPLICATE AQUARIUM: 25 YOUNG JUVENILES, 10 JUVENILES, 2 ADULTS.

Age Group	Mirex (mg/l)	Mortality (%) Observed Mean	S.D.	Range	Adjusted
Young juvenile 20-43 mm	acetone control	22.0	8.5	16 - 28	
	seawater control	22.0	2.8	20 - 24	
	0.01	27.0	10.5	16 - 36	6.4
	0.1	43.0	5.0	36 - 48	26.9
	1.0	47.0	5.0	40 - 52	32.1
	10.0	29.0	2.0	28 - 32	9.0
Juvenile 70-150 mm	acetone control	0			
	seawater control	0			
	0.01	0			0
	0.1	0			0
	1.0	0			0
	10.0	0			0
Adult 260-380 mm	acetone control	0			
	seawater control	0			
	0.01	0			0
	0.1	0			0
	1.0	0			0
	10.0	0			0

Table 3. CONCENTRATION OF MIREX ACCUMULATED BY STRIPED MULLET EXPOSED TO MIREX AND MEASURED CONCENTRATION IN WATER FOR 96 HOURS IN A CONTINUOUS-FLOW BIOASSAY. CONCENTRATION IN MULLET EXPRESSED AS WHOLE BODY WET WEIGHT. NUMBER OF FISH USED FOR ANALYSIS: 10 JUVENILES, 2 ADULTS PER REPLICATE AQUARIUM.

Age group	Mirex conc. (mg/l)		Mirex residue (μg/g)*	
	added	measured	mean	S.D.
Juvenile	0.01	0.010	0.17	0.02
	0.1	0.104	0.85	0.31
	1.0	1.108	3.90	0.16
	10.0	5.180	17.81	2.47
Adult	0.01	0.012	0.38	0.07
	0.1	0.107	1.02	0.17
	1.0	1.033	6.15	1.91
	10.0	9.540	37.30	4.83

*Duplicate samples per replicate were taken for analysis. Mean values were obtained with the data from eight separate analyses for each concentration.

Table 4. CONCENTRATION OF MIREX ACCUMULATED BY ADULT STRIPED MULLET (278-310 mm) EXPOSED TO 0.5 mg/l MIREX AND MEASURED CONCENTRATION IN WATER FOR 96 HOURS IN A CONTINUOUS-FLOW BIOASSAY. CONCENTRATIONS IN ORGANS EXPRESSED AS WET WEIGHT AND ARE FROM EIGHT ADULT FISH.

Exposure time (hr)	Measured conc. (mg/l)	Residue (μg/g)		
		skin, muscle	gills, heart	viscera
24	0.49	1.75	1.27	5.86
48	0.46	1.44	2.36	11.63
72	0.45	1.62	2.37	17.25
96	0.45	1.38	2.03	21.50

Methoxychlor was highly toxic to striped mullet. Young mullet were more susceptible than adults, and both age groups were more sensitive to methoxychlor than to mirex. Young juveniles exposed to 10.0 mg/l sustained 100 percent mortality within three hours of initial exposure while all adults exposed to the same concentration died within six hours. In 1.0 mg/l all juveniles were killed within nine hours and adults within 15 hours. At a concentration of 0.1 mg/l, about 95 percent mortality in the juveniles and 63 percent mortality in the adults occurred within 48 hours (Table 5). After 96 hours of exposure, mortality of adults and juveniles was nearly identical.

Table 5. PERCENT MORTALITY OF STRIPED MULLET EXPOSED TO METHOXYCHLOR FOR 96 HOURS IN A CONTINUOUS-FLOW BIOASSAY. FOUR REPLICATE AQUARIA WERE USED FOR EACH CONCENTRATION AND CONTROL. NUMBER OF FISH TESTED PER REPLICATE AQUARIUM: 25 YOUNG JUVENILES AND 2 ADULTS.

Age group	Methoxychlor conc. (mg/l)	Mortality (%)			
		Observed, mean		Adjusted	
		48-hr	96-hr	48-hr	96-hr
Young juvenile 20-43 mm	acetone control	23.0	25.0		
	seawater control	21.0	24.0		
	0.01	26.0	29.0	4.0	5.1
	0.1	96.0	98.0	94.8	97.4
	1.0	100.0	-	100.0	-
	10.0	100.0	-	100.0	-
Adult 260-380 mm	acetone control	0	0		
	seawater control	0	0		
	0.01	0	0	0	0
	0.1	62.5	100.0	62.5	100.0
	1.0	100.0	-	100.0	-
	10.0	100.0	-	100.0	-

During the experiments, affected mullet in the experimental tank showed behavior apparently caused by insecticide poisoning. This behavior included sudden random movements, attempts to jump out of the test tanks, gradual loss of equilibrium, and cessation of respiratory movements.

The concentrations of methoxychlor at which 50 percent of the test fish survived (TL_{50}) were estimated by the graphical method outlined by the American Public Health Association (1971). For young juvenile mullet, 48-hr and 96-hr TL_{50} values were 32 μg/l and 31 μg/l; for adult mullet the 48-hr and 96-hr values were 65 μg/l and 32 μg/l.

The toxicity study of methoxychlor on other fishes was made by Merna et al. (1972). They reported 96-hr continuous-flow TL_{50} of 7.5 μg/l for fathead minnow, Pimephales promelas, and 20.0 μg/l for yellow perch, Perca flavescens. A TL_{50} value of 40.0 μg/l for the yellow perch was found by Merna et al. (1972) for static toxicity tests. They attributed this higher value to the relatively more rapid breakdown of methoxychlor in static bioassay conditions. Eisler (1970) reported a 96-hr TL_{50} value of 63 μg/l for juvenile mullet, Mugil cephalus, from the east coast of the United States using a static bioassay technique.

Relatively small amounts of methoxychlor accumulated in the mullet (Table 6). Mullet exposed to 0.01 mg/l of methoxychlor for 96 hours concentrated about 0.1 μg/g in juveniles and 0.2 μg/g in adults. Accumulations of methoxychlor by the mullet exposed to the other concentrations were not significantly different for the same age groups. Juvenile and adult mullet exposed to 0.01 mg/l of methoxychlor in the accumulation test showed that skin and muscle, and gills and heart did not, in general, concentrate the insecticide as a function of exposure time while viscera did, even though the rates were very slow compared with those of mirex (Tables 7 and 8). Amounts of methoxychlor accumulated in visceral organs were approximately 0.3 μg/g for juveniles and 0.4 μg/g for adults after 96-hr exposure. However, the methoxychlor amounts found in other organs were not significantly different among the samples of each age, especially adults collected at 24-, 48-, 72- and 96-hr intervals after the initial exposure (0.01 μg/g for all skin and muscle and 0.12 μg/g or less for all gill and heart samples).

Table 6. CONCENTRATION OF METHOXYCHLOR ACCUMULATED BY STRIPED MULLET EXPOSED TO METHOXYCHLOR AND MEASURED CONCENTRATION IN WATER FOR 96 HOURS IN A CONTINUOUS-FLOW BIOASSAY. CONCENTRATION IN MULLET EXPRESSED AS WHOLE BODY WET WEIGHT. NUMBER OF FISH USED FOR ANALYSIS: 16 YOUNG JUVENILES, 2 ADULTS PER AQUARIUM.

Age group	Methoxychlor conc. (mg/l)		Methoxychlor residue (µg/g)*	
	added	measured	mean	S.D.
Young juvenile	0.01	0.009	0.06+	0.02
	0.1	0.104	1.64	0.57
	1.0	1.018	2.40	0.59
	10.0	9.361	1.69	0.30
Adult	0.01	0.010	0.20+	0.04
	0.1	0.108	11.92	2.00
	1.0	1.022	11.83	2.40
	10.0	9.690	11.12	2.72

* Duplicate samples per replicate were taken for analysis. Mean values were obtained with the data from eight separate analyses for each concentration.
+ Residue amounts in fish not killed by the insecticide.

Table 7. CONCENTRATION OF METHOXYCHLOR ACCUMULATED BY JUVENILE STRIPED MULLET EXPOSED TO 10 µg/l METHOXYCHLOR AND MEASURED CONCENTRATION IN WATER FOR 96 HOURS IN A CONTINUOUS-FLOW BIOASSAY. CONCENTRATION IN ORGANS EXPRESSED AS WET WEIGHT. NUMBER OF FISH USED FOR EACH ANALYSIS: 10 (75 - 134 mm STANDARD LENGTH).

Exposure time (hr)	Measured conc. (mg/l)	Residue (µg/g)		
		skin, muscle	gills, heart	viscera
0		ND*	ND	ND
8	9.7	0.01	0.02	0.06
24	9.9	0.03	0.10	0.10
48	9.5	0.01	0.03	0.15
96	9.1	0.02	0.11	0.27

*ND--none detected: less than 0.01 µg/g.

Table 8. CONCENTRATION OF METHOXYCHLOR ACCUMULATED BY ADULT STRIPED MULLET EXPOSED TO 10 μg/l METHOXYCHLOR AND MEASURED CONCENTRATION IN WATER FOR 96 HOURS IN A CONTINUOUS-FLOW BIO-ASSAY. CONCENTRATION IN ORGANS EXPRESSED AS WET WEIGHT. NUMBER OF FISH USED FOR ANALYSIS: 10 IN EACH AQUARIUM (310-381 mm STANDARD LENGTH).

Exposure time (hr)	Measured conc. (mg/l)	Residue (μg/g)		
		skin, muscle	gills, heart	viscera
24	11	0.01	0.12	0.24
48	14	0.01	0.10	0.11
72	11	0.01	0.10	0.17
96	12	0.01	0.12	0.39

Reinbold et al. (1971) in a study of uptake and metabolism of methoxychlor by some aquatic organisms found that the insecticide is readily metabolized in tilapia, Tilapia mossambica, and green sunfish, Lepomis cyanellus. The biodegradability of methoxychlor in aquatic organisms such as Gambusia affinis fish and mosquito larvae was also observed by Kapoor et al. (1970) and Metcalf et al. (1971).

SECTION IV

EFFECTS OF MIREX AND METHOXYCHLOR ON THE EGGS AND LARVAE OF STRIPED MULLET, Mugil cephalus L.

MATERIALS AND METHODS

Fertilized eggs and pro-larvae were available for experimentation following induced breeding of the adults in February 1974. Artificial spawning was induced in the laboratory by injecting salmon gonadotropin into the female mullet. After fertilization the eggs are usually incubated at 22°C in well-aerated and specially constructed seawater tanks.

In static acute bioassays (48 hrs for eggs, 96 hrs for larvae), eggs and larvae from the same female and male mullet were used. The test container adapted for the study was an 8" x 8" x 10" nylon net of Nitex #351 mesh immersed into a 20-gallon glass aquarium containing 55 liters of seawater. Desired amounts of the insecticide, previously dissolved in acetone (10 ml portion per tank), were added two hours before the eggs and larvae were introduced. Average temperature of the seawater measured throughout the bioassays was 22°C, with the range of 21.2 to 23.5°C; average salinity 32.5 °/₀₀; and average dissolved oxygen 6.4 ppm (range 4.5 - 7.2 ppm).

Once spawning occurred and fertilization was confirmed (95%), approximately 300 eggs (0.9 mm diameter) were transferred into each test container. Desired concentrations of mirex and methoxychlor were 0.01, 0.1, 1.0 and 10.0 µg/l, together with acetone and seawater control tanks. Three groups of 50 eggs each were taken daily from the individual test containers to estimate the percentage of dead embryos during 48-hour incubation bioassay. Viability was determined by microscopic examination and recorded on an Aristo hand tally counter. Developmental stages of live eggs were determined from illustration by Kuo et al. (1973).

Using an identical experimental system, a total of 300 pro-larvae (3 mm in length) was introduced into each container for 96-hour bioassay. Dead larvae were counted daily by three independent observers and the totals combined and averaged. Test water was sampled daily for insecticide analyses.

RESULTS

During the initial 12 hours of the egg bioassay, heavy mortalities occurred from all the test tanks including controls (Table 9). The mortality rates were

apparently higher in methoxychlor than in mirex throughout the test period. None of the eggs hatched during the period. Optimum hatching time has been determined by Kuo et al. (1973) to be 48 to 56 hours after fertilization at 20 - 24°C.

Table 9. PERCENT SURVIVAL OF STRIPED MULLET EGGS IN MIREX AND METHOXYCHLOR AND MEASURED CONCENTRATIONS OF BOTH INSECTICIDES IN TEST WATER FOR 48 HOURS IN A STATIC BIOASSAY. TESTS RUN SIMULTANEOUSLY FOR BOTH INSECTICIDES.

Insecticide	Concentration (mg/l)			Mean survival (%)		
		Measured				
	Added	24-hr	48-hr	12-hr	24-hr	48-hr
	acetone control		N.D.*	73	58	50
	seawater control		N.D.	80	80	64
Mirex	0.01	0.008	0.006	43	24	23
	0.1	0.091	0.062	25	23	23
	1.0	0.584	0.435	18	14	13
	10.0	4.277	3.366	29	24	14
Methoxychlor	0.01	0.009	0.007	14	11	10
	0.1	0.083	0.070	19	12	7
	1.0	0.848	0.612	9	8	7
	10.0	8.273	7.683	14	9	7

*N.D.--none detected: less than 0.1 μg/l mirex, 0.08 μg/l methoxychlor.

The measured insecticide concentrations in the test water (Tables 6 and 7) varied. Concentrations measured from the fourth-day samples collected from the larval bioassay tanks decreased rapidly compared with those from the first-day samples for all levels; and the higher the added concentrations, the greater the decreasing rate observed in both mirex and methoxychlor test water. This was probably due to the low solubility, adsorption by apparatus, uptake by test organisms, volatilization, rapid breakdown, etc., of the insecticides in the standing seawater.

It was apparent that methoxychlor was more toxic to mullet larvae than mirex

(Table 10). Larval mortalities in 10 mg/l methoxychlor test water on the first and second day were 95 and 100 percent, respectively, compared with 24 and 52 percent in the same concentration of mirex test water. Representative tolerance limit values of both insecticides for mullet larvae were not obtained from this study. The unexpectedly severe mortalities observed from the acetone control tanks prevented application of statistical testing.

Table 10. PERCENT SURVIVAL OF STRIPED MULLET LARVAE IN MIREX AND METHOXYCHLOR AND MEASURED CONCENTRATION OF BOTH INSECTICIDES FOR 96 HOURS IN A STATIC BIOASSAY. BIOASSAY WAS MADE SIMULTANEOUSLY FOR BOTH INSECTICIDES.

Insecticide	Concentration (mg/l)					Mean survival (%)			
	Added	Measured							
		24-hr	48-hr	72-hr	96-hr	24-hr	48-hr	72-hr	96-hr
	acetone control				N.D.*	87	70	56	55
	seawater control				N.D.	96	92	82	77
Mirex	0.01	0.009	0.008	0.007	0.006	94	89	86	77
	0.1	0.158	0.102	0.080	0.062	92	85	83	75
	1.0	0.835	0.867	0.569	0.389	85	84	81	69
	10.0	6.811	5.489	3.322	2.238	76	48	46	45
Methoxychlor	0.01	0.011	0.007	0.008	0.007	93	69	60	41
	0.1	0.133	0.163	0.133	0.266	70	53	50	38
	1.0	0.736	0.823	0.531	0.238	49	1	0	-
	10.0	8.268	8.386	5.210	1.260	5	0	-	-

*N.D.--none detectable: less than 0.1 µg/l mirex, 0.08 µg/l methoxychlor.

The culture of marine and brackishwater organisms is still very much at a developmental stage. Incubation of eggs and rearing the larvae in static and/or non-aerated conditions, in general, increases mortality. Furthermore, differences in the quality of the eggs from individual broodstock are being demonstrated by biochemical analyses. Consequently there are inherent differences in the viability of each larval population. A fertilized egg produced by induced spawning methods as yet does not guarantee a viable larva. In order to evaluate effectively the susceptibility of eggs and larvae to any pesticide, several tests are necessary with the progeny of different broodstock, and preferably with the same broodstock in subsequent breeding seasons.

SECTION V

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